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Note

Separation of octanoic acid and thiaoctanoic acid analogues by gas–liquid and high-performance liquid chromatography

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In the course of studies on medium-chain acyl-CoA synthetase [acid: CoA ligase (AMP); E.C. 6.2.1.2] we have directed our attention to the substrate specificity properties. Since this enzyme is notable for its relatively low specificity (in fact it activates C_4–C_{12} straight-chain fatty acids with maximal activity toward octanoic acid) we proposed to determine whether this system for ATP-acylate activation could use sulphur analogues of fatty acids[1].

In view of the increasing knowledge of the "ambiguity phenomenon" of enzymology of sulphur-containing compounds[2], we first describe the syntheses and gas chromatographic (GC) or high-performance liquid chromatographic (HPLC) separation of analogues of octanoic acid in which a methylene carbon is substituted with a sulphur atom at positions 3, 4, 5 or 6 respectively:

$COOH–(CH_2)_6–CH_3$	Octanoic acid
$COOH–CH_2–S–(CH_2)_4–CH_3$	3-Thiaoctanoic acid
$COOH–(CH_2)_2–S–(CH_2)_3–CH_3$	4-Thiaoctanoic acid
$COOH–(CH_2)_3–S–(CH_2)_2–CH_3$	5-Thiaoctanoic acid
$COOH–(CH_2)_4–S–CH_2–CH_3$	6-Thiaoctanoic acid

MATERIALS AND METHODS

The same general method was used for the synthesis of all the *n*-alkyl-thia-acid analogues of octanoic acid, for brevity termed thiaoctanoic acids.

3-Thiaoctanoic acid (*n*-pentylthioglycolic acid) was prepared by reaction between thioglycolic acid and pentyl bromide. 4-Thiaoctanoic acid (3-*n*-butylthiopropionic acid) was synthesized from 3-bromopropionic acid and 1-butanethiol; 5-thiaoctanoic acid (4-*n*-propylthiobutyric acid) was prepared by using 4-chlorobutyric acid and 1-propanethiol, while for the synthesis of 6-thiaoctanoic acid (5-ethylthiovaleric acid) we used ethanethiol and 5-bromovaleric acid. All chemicals were of the best available grade from Fluka (Buchs, Switzerland).

0.10 mole of the bromo or chloro derivative and 0.11 mole of the thiol compound were dissolved in 100 ml of 90 % ethanol containing 0.3 *M* KOH. After boiling under reflux for 3 h, 150 ml of water were added and ethanol and volatile unreacted

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compounds were distilled off. The aqueous solution was acidified to pH 1.0 with concentrated HCl, liberating the desired acid as a clear oil. This was extracted three times with 50 ml of diethyl ether, dried with anhydrous sodium sulphate and the ether distilled off.

GLC analyses were performed with a Perkin-Elmer Model 990 instrument, equipped with a flame ionization detector, using a 3 m × 3 mm I.D. glass column filled with 10% OV-17 supported on Chromosorb W HP DMCS (100–120 mesh). Flow-rates were: 15 ml/min for nitrogen carrier gas; 20 ml/min for hydrogen and 300 ml/min for air. Temperatures were 150°C for the column and 220°C for the injector and detector. Methyl esters of octanoic and thiaoctanoic acids were obtained by treatment with diazomethane[3].

For HPLC analyses a Spectra-Physics liquid chromatograph equipped with SP 8700 solvent-delivery system, SP 8750 Organizer, Model 770 spectrophotometric detector and SP 4100 computing integrator was used with a prepacked RP-18 column, size 10 μm. The column was equilibrated with acetonitrile–water (53:47 v/v) acidified at pH 3.03 with phosphoric acid, and the same solvent was used as mobile phase. Analyses were performed at room temperature at a pressure of 290 p.s.i. with a flow-rate of 0.8 ml/min. The wavelength for detection was 210 nm.

RESULTS AND DISCUSSION

In Fig. 1 are shown the results of the GLC separation of octanoic and thiaoctanoic acid methyl esters. Under the conditions employed the five compounds are well separated and the retention times were respectively 11.2, 37.6, 40.0, 43.4 and 49.6 min for the methyl esters of octanoic acid and 3-, 4-, 5- and 6-thiaoctanoic acids. It is interesting to note the different retention times of the thiaoctanoic methyl esters

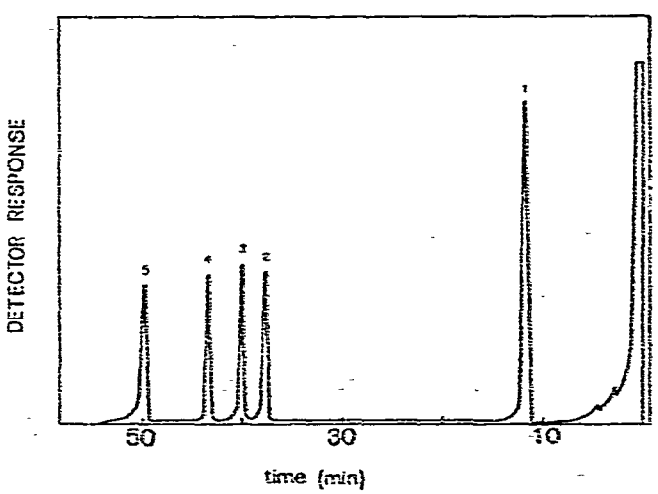

Fig. 1. Gas chromatogram of 10 μg of methyl esters of octanoic and thiaoctanoic acids. Peaks: 1 = octanoic acid methyl ester; 2 = 3-thiaoctanoic acid methyl ester; 3 = 4-thiaoctanoic acid methyl ester; 4 = 5-thiaoctanoic acid methyl ester; 5 = 6-thiaoctanoic acid methyl ester.

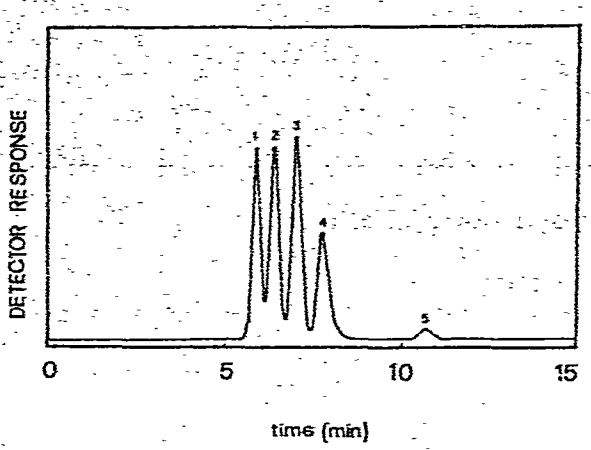

Fig. 2. Separation of 5 μg underivatized octanoic and thiaoctanoic acids by HPLC. Peaks: 1 = 6-thiaoctanoic acid; 2 = 5-thiaoctanoic acid; 3 = 4-thiaoctanoic acid; 4 = 3-thiaoctanoic acid; 5 = octanoic acid.

indicating their different volatilities compared to octanoic acid and which are dependent on the position of the sulphur atom in the carbon chain.

This GLC behaviour of the methyl esters prompted us to utilize also HPLC for the separation of the underivatized compounds. In Fig. 2 are reported the results of HPLC analyses using the reversed-phase RP-18 column. The various polarities of the compounds, decreasing from 6-thiaoctanoic acid to octanoic acid, result in a good separation of each component of the mixture. The difference between the absorbance of octanoic acid and that of the thia-analogues, at 210 nm, is due to the presence in the latter of the thioether linkage.

Thus we have developed a ready method for the synthesis and rapid analysis of thia-analogues of octanoic acid, giving an useful system for the study of the metabolic interactions between octanoic acid and its sulphur-containing analogues in biological systems involving the transformation of fatty acids.

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